out among our patients. Before being allocated into the study groups, the patients had undergone a course of dental treatment programs for three weeks in order to reduce the amount of superimposed dental inflammation as much as possible. Thereafter, the patients were randomized into intervention and control groups. Metronidazole tablets 250 mg/TDS were given to the intervention group, while the control group received the same amount of placebo. Patients were examined by a blinded periodontologist, at days 0, 7, 14, and 21, to determine the gingival overgrowth scores. **Results**: The two groups were identical in age, gender, cause of receiving cyclosporine (kidney or bone marrow transplantation), duration of receiving cyclosporine, plasma level of cyclosporine and gingival overgrowth scores. Gingival enlargement improved in 7 patients, of whom 6 were in our intervention group (54.5%). The response to treatment was different in two groups significantly (P= 0.03). No association was found between gender, cause of receiving cyclosporine, duration of receiving cyclosporine, plasma level of cyclosporine and gingival overgrowth scores variables and response to treatment. **Conclusion**: In conclusion, our findings suggest that we can benefit the advantages of metronidazole administration in CIGH. Although we think that higher doses of metronidazole in association with oral hygiene programs and dental treatment planning could show better results in such patients.

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RECOVERY OF LEUKOCYTES FROM CORD BLOOD UNITS AFTER CONTROLLED RATE FREEZE IN DMSO AND CRYOPRESERVATION IN THE VAPOR PHASE OF LIQUID NITROGEN

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American Red Cross, Western Area Community Cord Blood Bank has established a program to assure the quality of cryopreserved volunteer donor cord blood units (CBU). Recovery (Rec) of leukocytes (WBC) and WBC viability after CBU thawing are important surrogate measures CBU quality. We determined the Rec of WBC in cryopreserved CBU immediately post-thaw and after dilution and washing in Dextran 40/5% Albumin (D/A), which is widely employed. Methods: CBU were collected from consented volunteer donors. CBU that were eligible for transplant, but had less WBC than required by storage criteria (6 E8) were studied. CBU were processed within 48 hours by Rubinstein's method. The CBU volume after addition of 10% DMSO was 25 ml. CBU were cryopreserved by controlled rate freezing and stored in vapor phase liquid nitrogen for up to 1 year. Twelve CBU selected for study were removed from storage and immediately placed in a 37 C waterbath. After thawing, the CBU was maintained at 4 C and a sample was immediately removed to assess post-thaw WBC (% NC-Rec) and cell viability by Trypan blue dye exclusion (% TB-viable). The CBU was diluted over 2 min in 25 ml D/A, samples removed, then the CBU was diluted to 100 ml in D/A. The CBU was centrifuged at 400 xG at 4 C and resuspended in 25 ml of D/A. WBC Rec and NC viability were assessed post-thaw, after the 1:1 dilution in D/A, and post wash and resuspension. In separate experiments, the Rec of CD34-bearing cells was measured. Results: There was a median 22% loss of NC after thawing, and additional NC loss after dilution and washing. Post thaw trypan blue dye viability was slightly reduced. In 6 separate experiments the Rec of CD34+ cells was 107 ± 31% after thawing and 106 ± 33 after washing. Conclusions: Thawing of cryopreserved CBU was associated with loss of approximately 22% of the total NC and reduction in overall cell viability. Further cell loss accompanied washing. Recovery of CD34 bearing cells was superior to total NC. Hematopoietic progenitor growth in culture after CBU thawing was consistent with the above.

Time	Post Thaw	Post Thaw	Post Dilution	Post Dilution	Post Wash	Post Wash
Data	% NC Rec	% TB viable	% NC Rec	% TB viable	% NC Rec	% TB viable
Mean ± SD	78±12	90±6	76±12	90±8	65±18	90±7
p	0.02	0.002	0.019	0.014	0.001	0.005

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EX VIVO ENGINEERING OF PREVIOUSLY THAWED AND CRYOPRESERVED UMBILICAL CORD BLOOD (UCB) WITH INTERLEUKIN (IL)-2, IL-7, IL-12 AND ANTI-CD3 FOR EXPANSION OF CYTOTOXIC T LYMPHOCYTES (CTL): PROMISING STRATEGY FOR ADOPTIVE CELLULAR IMMUNOTHERAPY (ACI) POST UCB TRANSPLANTATION(T)

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Limitations associated with using UCB as a source for ACI post UCBT includes the lack of donor immunoeffector cells from the original cryopreserved UCB unit and/or immaturity of CB cellular immunity. We have demonstrated that CTL can be selectively engineered and activated from fresh and cryopreserved and thawed (CT) aliquots of UCB (Robinson/Cairo et al, Exp Hem 30:245, 2002). In this study we evaluated and compared the activation and NK and LAK cytotoxicity of UCB CTL. Thawed UCB aliquots were monocyte depleted (5 x10^6 cells/ml) in serum-free (SF) AIM-V in 5% CO_2 @ 37°C. Nonadherent cells (1x10^6 cells/ml) were either cultured in SF AIM-V + anti-CD3 (50 ng/ml), IL-2 (5 ng/ml), IL-7 (10 ng/ml) and IL-12 (10 ng/ml) (AB/CY) or in SF AIM-V alone for 48 hours @ 37°C in 5% CO_2 or expanded, recryopreserved and rethawed (TECT), or not expanded but recryopreserved, rethawed and subsequently expanded (TCTE). NK subsets were analyzed by flow cytometry and NK and LAK cytotoxicity by WST-1 methodology utilizing a 10:1 E:T ratio against K562 (NK) and Daudi (LAK), respectively. A significant enhancement in NK cytotoxicity was seen when UCB cells were cultured in the AB/CY cocktail ($p<0.001$) when compared to media alone, but no difference between the modalities (TE: 0.73±0.03 vs 0.16±0.01; TECT:0.72±0.03 vs 0.16±0.01 and TCTE: 0.75±0.04 vs 0.16±0.01). Similarly, there was significant enhancement of LAK cytotoxcity with all modalities of AB/CY stimulation vs media alone ($p<0.001$) but no difference between modality (TE: 0.39±0.01 vs 0.26±0.01; TECT: 0.37±0.008 vs 0.23±0.002; TCTE: 0.41±0.01 vs 0.20±0.01). Furthermore, there was a significant increase in the CD3-/16+/56+ subset of TE, TECT and TCTE when compared to media alone (TE: 60.2±1.24% vs 47.33±0.55%, n=3, $p<0.001$; TECT: 60.67±2.4% vs 46.68±1.3%, n=3, $p<0.001$; TCTE: 60.7±3.27% vs 45.45±1.4%, n=3, $p<0.001$). These data suggest that previously cryopreserved and thawed UCB aliquots may be engineered at time of UCB transplant, ex vivo expanded and activated for cytotoxic (NK & LAK) potential and recryopreserved for later use for DLI post UCBT. Xenotransplant animal studies are underway to examine the in vivo effects of this UCB CTL population.

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ONCE AND TWICE FILGRASTIM FOR ALLOGENEIC PBSC MOBILIZATION

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Objective: To compare efficacy and safety in allogeneic peripheral blood stem cell (PBSC) donors who received 400μg/m^2/day of filgrastim administered subcutaneously once daily (Once-daily regimen) or in two-divided dose every 12 hours (Twice-daily regimen). Design: An open-label, randomized, multicenter phase III trial. Patients: Between May 2001 and May 2002, 72 PBSC donors were enrolled in this study. Eligibility criteria was as follows: Allogeneic PBSC donors to related patients aged between 16 and 65 years, eligible for the criteria described in the Japan Society for Hematopoietic Cell Transplantation (JSHCT) guideline of PBSC harvest. Intervention: Donors were randomly assigned to receive 400μg/m^2 of G-CSF (filgrastim) subcutaneously once daily or in two-divided doses every 12 hours on 3 consecutive days. PBSC was collected by leukapheresis using COBE Spectra on day 4. Main Outcome Measures: The primary endpoint of this study was CD34 positive cell dose per kg of donor's body weight collected by leukapheresis on day 4. Additional endpoints were total nucleated cell (TNC) counts in the leukapheresis products, total pain burden experienced by donor measured with area under curve (AUC) of visual analogue scale (VAS), and total dose of analgesic drugs. Safety endpoints: Incidence and severity of

adverse events. Main Results: The number of CD34+ cells in the leukapheresis products were similar in the two groups: the geometric mean number of CD34+ cells was 1.73 x 10^6 /kg in the once-daily group and 2.08 x 10^6 /kg in the divided-dose group (P=.0.37). The geometric mean number of TNCs in the products was 6.43 x 10^8 /kg in the once-daily group and 6.35 x 10^8 /kg in the twice-daily group (P=0.87). Although the median total doses of analgesic drug were similar in both groups (6.35mg/kg vs 7.69mg/kg acetoaminophen, P=0.79), the donors in the once-daily group experienced significantly less pain than twice-daily group (median AUS of VAS; 21.5 vs 52.5 mm*days, P=0.043). The incidences and severities of adverse events other than pain were similar in both groups. Conclusions: In comparison with two-divided dose regimen, once-daily injection of 400μg/m^2 of G-CSF is equally effective for allogeneic PBSC mobilization and associated with less pain. Taking into account of donor's convenience, once-daily regimen is preferable to twice-daily regimen.

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IN VITRO PRIMING AND EXPANSION OF CMV-SPECIFIC TH1 AND TC1 T CELLS FROM NAIVE CORD BLOOD LYMPHOCYTES BY AUTOLOGOUS DENDRITIC CELLS

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BACKGROUND: Adoptive transfer of CMV specific cytotoxic T cells (CTL) expanded in vitro from donor memory cells have reduced CMV disease in the allogeneic BMT setting. However, this approach has not been feasible in the unrelated cord blood transplant (uUCBT) setting as cord blood contains naive, unprimed T cells. We developed an in vitro protocol to prime and expand CMV-specific CTL from cord blood. METHODS: Monocytes from 6 UCB units were cultured with GM-CSF/ IL-4 for 6 days to generate immature DCs (Mo/DCs). Autologous UCB lymphocytes were primed either with CMV-lysate-pulsed Mo/DCs (CMV+) or as negative controls unpulsed (CMV-) or sham lysate loaded Mo/DCs. Prior to priming CMV +/- loaded immature DC received 12h maturation signals (TNFα+PGE2) inducing expression of CD80, CD86 and HLA molecules. For restimulation and expansion of CTL cultures DC were generated from CD34+ cells cultured in SCF/Flt-3L /GM-CSF /IL-4 for 14 ~ 21days and GM-CSF/IL-4 for 6days. DCs and responder lymphocytes were cultured in the presence of low dose IL-2 (10IU/ml), IL-7, and IL-12 to skew the priming and expansion towards Th1 and Tc1 cells. CTL cultures were re-stimulated weekly for 5~ 7 weeks. After the 4th week CD3+ T cells were tested weekly by ic FACS analysis for CMV-specific IFNγ, TNFα secretion. CMV-specific cytotoxicity was tested by LDH release assay on CMV+, CMV- or sham lysate loaded autologous Mo/DC. RESULTS: CMV+ cultures expanded significantly more (3.5 ~ 13.39 fold) than CMV- cultures (0.65 ~ 7.8 fold) despite identical cytokine milieu. There was a preferential expansion of CD45RA-/RO+ 'memory' T cells in CMV+ cultures compared to CMV- cultures (CMV+: 66~86% of CD3+ versus CMV-: 30 ~ 46% of CD3+. CMV-specific interferon gamma producing T cells were 4.7 ~ 35.3% (mean: 16.1%) in CMV+ cultures and 0 ~ 2% (mean 0.5%) of CD3+ in CMV- cultures respectively (p=0.025) with both CD4+ (Th1) and CD8+ (Tc1) T cells contributing. CMV-specific cytotoxicity at 10:1 E:T ratio was 31 ~ 48 % from CMV+ cultures and 10~ 13% from CMV- or sham lysate cultures. CONCLUSION: This dataset is the first to our knowledge to demonstrate the generation of CMV-specific CTLs that were primed and expanded in vitro from naive umbilical cord lymphocytes. This approach may lead to effective clinical strategies against CMV infection in the uUCB transplantation setting.

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UPREGULATION OF THE EXPRESSION OF MOLECULES INVOLVED IN ALLORECOGNITION: IMPLICATIONS FOR POTENTIATING THE MIXED LYMPHOCYTE REACTION PRIOR TO SELECTIVE ALLOREACTIVE T CELL DEPLETION

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Various mechanisms of selective depletion of alloreactive T cells in haematopoietic stem cell (HSC)grafts have been described: all depend upon the stimulation of donor alloreactive cells by recipient cells in a unidirectional mixed lymphocyte reaction (MLR). In the HLA identical donor setting, stimulation of alloreactive donor cells in a standard MLR often fails to occur: in this setting a modification of the MLR with cytokine pre-treatment of stimulator cells has been described. We investigated the effect of two strategies designed to potentiate the MLR on expression of cell surface molecules important in allostimulation. The expression of adhesion molecules (CD11a, CD54(ICAM 1)), co-stimulatory molecules (CD80 and CD86) and HLA class I and Class II molecules were measured by flow cytometry after stimulation of monocytes and T cells with TNF-alpha, IL4 and/or gamma-interferon for 24 and 48 hours. Similar observations were made of T cells stimulated with immobilized mitogenic OKT3 +/- agonistic anti-CD28. The expression of CD178 (Fas ligand) in these cells was also determined at 24 and 48 hours. Effective upregulation of molecules involved in allorecognition occurred with all three cytokines on monocytes and with g-IFN and TNF-a on T cells. Use of immobilised OKT3 caused much greater increases in expression of similar molecules on T cells and an increase in the proportion of T cells expressing FAS ligand. The addition of anti-CD28 to OKT3 had no additional benefit in upregulation of these molecules. Results are summarised below (NT=Not Tested). Both strategies may enhance alloantigen presentation in the HLA identical situation and facilitate the activation (and subsequent removal) of alloreactive cells in HSC grafts. The increase in the proportion of T cells expressing FAS ligand seen with OKT3 stimulation may enhance FAS-mediated apoptosis of alloreactive cells. We are currently investigating the use of both techniques in modifications of the MLR as part of our selective allodepletion research programme.

Treatment	IL-4		Gamma-Interferon		TNF-Alpha		OKT3
	CD3+ Cells	CD14+ Cells	CD3+ Cells	CD14+ Cells	CD3+ Cells	CD14+ Cells	CD3+ Cells
CD11a	↔	↔	↔	↔	↔	↔	↑
CD54	↔	↔	↑	↑↑	↔	↑↑	↑↑↑
CD80/86	↔	↔	↔	↑	↔	↔	↑↑
HLA Class I	↔	↑	↑	↑	↑	↑	NT
HLA Class 2	↔	↑	↔	↑↑	↔	↑	↑↑↑
CD178	NT	NT	NT	NT	NT	NT	↑↑

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TIME-TEMPERATURE STUDY OF CELL SURVIVAL IN BONE MARROW (BM) AND BLOOD HEMATOPOIETIC PROGENITOR CELL (HPC) GRAFTS

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Background: Standard practice in BM and blood HPC transplants facilitated by the NMDP is to ship BM HPC grafts at room temperature and blood HPC grafts at 4°C. There is limited information regarding the effect of storage conditions on the content of cell subsets in the graft. We undertook a prospective study of the effect of time and temperature on the content of viable CD34+ cells, T-cell, B-cell, NK cells and dendritic cell subsets in blood and BM HPC grafts. Method: Small aliquots of blood (N=4) and BM (N=5) HPC grafts from patients harvested at two transplant centers were obtained from quality control samples discarded from the processing laboratories. The number of viable nucleated cells was determined by fluorescent microscopy following staining with ethidium bromide and acridine orange and storage for 24, 48 or 72 hours at 4°C or RT. The content of CD34+ cells, T-cells, B-cells, NK cells and dendritic cell subsets was determined using flow cytometry and propidium iodide to electronically exclude non-viable cells. Results: BM grafts had better overall cell viability during storage than blood HPC grafts at both temperatures. The median number of viable CD34+ cells in BM HPC grafts was stable at 4°C and RT during 48 hours storage then declined to 50% and 29% of the initial values, respectively, after 72 hours. In contrast, there was a steady decline in the numbers of viable CD34+ cells in blood HPC grafts throughout